Phytochemical composition of everbearing strawberries and storage quality of strawberry fruit treated by precooling

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Abstract This study was conducted to investigate the nutrient composition of everbearing strawberry cultivar grown on highlands of Gangwon province after harvest. Various phytochemical compositions such as ascorbic acid, anthocyanin, malondialdehyde, and phenolic compounds were analyzed in strawberry fruits from five different cultivars (cv. Albion, Flamenco, Goha, San Andreas, and Seascope). Ascorbic acid content was similar among five cultivars at 20 mg 100 g⁻¹, whereas major pigment anthocyanin in Seascope was higher than in any other cultivars. Goha and San Andreas showed higher total phenolic compound contents. The effect of room or forced-air precooling on Flamenco and Goha was studied. Higher weight loss was observed in Flamenco treated with forced-air cooling, while in Goha both precooling treatments induced weight loss. Both precooling treatments were effective to maintain firmness in two strawberry cultivars. Forced air cooling maintained total phenolic contents until 12 days of storage, and the efficiency accelerated by low temperature storage.

Keywords Anthocyanins · Nutritional value · Total phenolic compound

Introduction

Most of the horticultural products contain a lot of antioxidants and phytochemicals, which act to inhibit the aging of humans and to reduce the incidence of diseases such as infections, immune system disorders, and cancer (Cai et al., 2004; Eum et al., 2009; Roleira et al., 2015). Strawberry is a major horticultural product consumed worldwide because of its unique fragrance and high nutritional value. According to the Food and Agriculture Organization of the United Nations, the worldwide area of cultivation in 2016 was 402,000 ha, 1.5 times more than in the past 20 years, while strawberry production increased 2.5 times in 2016 to 9.1 million tons, about 80% of the strawberries distributed in the market are consumed as fresh fruit whereas the remaining 20% is used for processing (FAOSTAT, 2016; Ornelas-Paz et al., 2013; Šamec et al., 2016). The high antioxidative activity of strawberry is mainly attributed by vitamin C and polyphenol components, and major polyphenol components are anthocyanins, ellagic acid derivatives, and flavonols (Erkan et al., 2008; Giampieri et al., 2012; Tudor et al., 2015; Wang et al., 1996; Wang and Zheng, 2001).

Strawberry cultivation in Gangwon-do is a cultural practice in highland during summers and in low land during winters, a climate condition capable of year-round production of the strawberries. The summer strawberry is an everbearing strawberry and its harvest time is from June to November. The major production areas are highlands of Pyeongchang and Yanggu in Gangwon province. The representative summer strawberry cultivars cultivated in the highlands are Flamenco, Charlotte, Goha, and Albion. The nutritional value of strawberries is mostly concentrated on June-bearing strawberries in winters and the data on everbearing strawberries in summers are insufficient.

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Strawberries have a higher respiration rate than other crops after harvesting, and when left at room temperatures in summer, freshness is rapidly decreased due to high respiration heat (Li and Kader, 1989; Martinez-Romero et al., 2003; Nunes et al., 1995a; 1995b; Pérez et al., 1998). Therefore, it is a major attribute to maintain the fruit quality by quickly lowering the respiration heat generated after harvesting the strawberries. A typical pretreatment technique before storage to remove the respiratory heat after harvesting the fruit is precooling. The precooling treatment that can be treated on the strawberry is room precooling and forced-air precooling, and precooling treatment has different effects on quality during distribution depending on the cultivar (Eum et al., 2014).

In this study, the functional components by different everbearing strawberry cultivars cultivated in highlands were investigated and the effects of the precooling on maintaining quality during cold storage for the major strawberry cultivars were also investigated.

Materials and methods

Plant materials and treatment conditions

The everbearing strawberries cultivated in the Poongsung farm located at 700 m highlands in Daegwallyeong, Pyeongchang-gun, Gangwon-do were supplied in September 2013. The five everbearing strawberries, Albion, Flamenco, Goha, San Andreas, and Seascape, were also tested. The strawberry cultivars used in this study were Goha, bred in Korea, and imported Albion, Flamenco, San Andreas, and Seascape. Conventionally strawberries with 90% coloring were harvested and used in this study.

Among these, two cultivars of Flamenco and Goha were room precooled and forced-air precooled at 0 °C until 7/8 cooling time. Room precooling was carried out in the cold storage chamber at 0 °C, while forced-air cooling was carried out by keeping each 50 everbearing strawberries in the plastic box (34 cm × 26 cm × 5.5 cm). Each plastic box was stacked with each six (2 pieces × 3 pieces) in four layers and forced-air precooling was carried out after covering the entire stack with a vinyl using a simple forced-air precooling device (FOX-S1004, DSFOX, Korea) under a wind velocity of $3.1 \times 10^{-3} \text{ m}^3 \text{ s}^{-1} \text{ kg}^{-1}$. Temperature of the fruit flesh was 26 °C which was similar to the outer air, and the time consumed till 7/8 cooling time at the low temperature storage room at 0 °C was around one and half hour. The precooled strawberries were packed in the paper boxes that are used during distribution and stored in the storage room at 4 °C to monitor the quality change.

Quality evaluation

The weight change was expressed as a percentage of the initial weight loss over the storage period. Soluble solid content (SSC) of stored strawberries were measured by refractometer (PAL-1, Atago, Tokyo, Japan) by extracting juice and expressed as °Brix. The acidity was measured with a pH meter (SAM-706AC, G-won Hightech Co., Seoul, Korea) and expressed as % in terms of citric acid. The measured sugar content and acidity value were used to express the sugar acid ratio. Firmness of the fruit was measured at the equatorial region of the strawberries with a texture analyzer (EZ Test/CE-500 N, Shimadzu, Kyoto, Japan) under 120 mm min⁻¹ crosshead speed condition by Ø 3 mm probe, and expressed as newton (N). The chromaticity of the strawberries was expressed as CIE L*, hue angle, and chroma value by measuring the two equatorial regions of the strawberry peel with a colorimeter (CR-400, Minolta, Osaka, Japan).

Analysis of major compositions

Ascorbic acid contents of the fruits were measured by homogenizing 5 g of fruit flesh with 2% metaphosphoric acid and 2 mM EDTA solution, and the mass was centrifuged. The supernatant was filtered with 0.45 µm syringe, and ascorbic acid contents were measured with HPLC (YL9100, Young-Lin, Anyang, Korea). HPLC measurement was carried out at 254 nm with a mobile phase of 0.1 M sodium phosphate and 0.2 mM EDTA (pH 3.1) under isocratic condition (flow rate at 0.7 mL min⁻¹). Microbondapak C₁₈ reverse phase column (3.9 × 300 mm; Waters Corp., MA, USA) was used during analysis.

Anthocyanin contents were measured with pH differential method as in AOAC (2010). 2 g of strawberry flesh was homogenized using 0.1% hydrochloric acid methanol solution and then this mass was centrifuged. The supernatant was mixed with a solution of pH 1.0 and pH 4.5. The mixture was determined for anthocyanin contents using spectrophotometer (Specord 40, Analytik jena, German) at 520 and 700 nm, respectively. Anthocyanin contents were determined using the equation below.

Meanwhile, the pH of each prepared solution was calibrated by titrating 25 M potassium chloride with hydrochloric acid until the pH of the solution became 1.0 for the pH 1.0 buffer. For preparing the pH 4.5 buffer, 0.4 M sodium acetate solution was titrated using hydrochloric acid until the pH of solution became 4.5.

$$A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}} \\ (A \times \text{MW} \times \text{DF} \times 1000) / (\varepsilon \times 1)$$

where MV = 449.2 and ε = 26,900.

Fig. 1 Ascorbic acid (A) and anthocyanin content (B) in fruits of everbearing strawberries grown on highland (700 m above sea level) in Pyeongchang area in 2013. The data presented the mean $\pm$ SE (n = 10). The different normal letters indicate significant differences at 0.05 level (Duncan's multiple range test)

Total phenolic compound was determined using Folin–Ciocalteu solution. 2 g of strawberry flesh was homogenized using 80% acetone solution in which 0.2% formic acid was contained. 6 mL distilled water, 0.5 mL Folin–Ciocalteu reagent, and 1.5 mL 20% Na₂CO₃ were added into 1 mL of centrifugal supernatant and mixed by adding distilled water to make the final volume 10 mL. Mixture was left at 70 °C for 10 min, and then phenolic acid contents were measured using spectrophotometer at 765 nm. Gallic acid was adopted as a standard during analysis (Xu et al., 2016).

Malondialdehyde was determined by thiobarbituric acid method (Kaniuga and Michalski, 1978). 2 g of strawberry flesh was homogenized using 0.2 M phosphate buffer solution in which 1% Triton X-100 was contained. 1 mL of centrifuged supernatant was mixed with 20% trichloroacetic acid solution in which 0.5% TBA was contained. The mixture was left at 95 °C for 30 min, and then centrifugation was carried out once more. Malondialdehyde was determined using spectrophotometer at 532 nm

Fig. 2 Total phenolic content (TPC, A) and MDA production (B) in fruits of everbearing strawberries grown on highland (700 m above sea level) in Pyeongchang area in 2013. The data presented the mean $\pm$ SE (n = 10). The different normal letters indicate significant differences at 0.05 level (Duncan's multiple range test)

and 600 nm, respectively. MDA concentration was quantified using equation $E = 155 \text{ mM cm}^{-1}$.

Results and discussion

Comparison of nutritional composition by everbearing strawberry cultivars

The content of ascorbic acid, known as antioxidant, was about 20 mg 100 g⁻¹ in all everbearing strawberry cultivars and showed similar contents (Fig. 1A). It was reported that the color of strawberry is mainly determined by the accumulation of anthocyanin and polyphenolic compounds. Major polyphenolic compounds in the strawberries are flavan-3-ols, ellagitannins, cinnamic acid conjugates, flavanols, and ellagic acid conjugates (Šamec et al., 2016). Total anthocyanin content of strawberry, which is a major color of the fruit, was 12.5 mg 100 g⁻¹, which was the highest in Seascape, followed by San Andreas, Albion,

Fig. 3 Change in weight loss (**A**, Flamenco; **B**, Goha) and firmness (**C**, Flamenco; **D**, Goha) of everbearing strawberries during storage at low temperature (4 °C). The data presented the mean $\pm$ SE (n = 5).

Flamenco, and Goha (Fig. 1B). According to Šamec et al. (2016), color change of strawberry is dominated by genotype rather than cultivation environment. On the other hand, Goha showed the lowest color change trend. It was because Goha was the least colored at harvest time. Generally, the time of harvesting everbearing strawberry at the farm is, in the case of Goha, when coloration progresses to 50–60% by the farms compared to other cultivars. Previous research results show that the firmness of Goha harvested when coloration progressed to 50–60% was 1.5 N, and SSC was around 7° Brix, by which the quality level was similar with other everbearing strawberry cultivars (Eum et al., 2014). When fruits with much coloring were harvested, the softening speed was accelerated. Therefore, strawberries were harvested in a colored state at the farms in coloration progressed to 50–60%.

Total phenolic compound was the highest in Goha and San Andreas, among the five cultivars tested, and it was in a similar level in Albion, Flamenco, and Seascape. Reactive oxygen species (ROS) decompose unsaturated fatty acids present in the cell membrane to produce the final

The different normal letters indicate significant differences at 0.05 level (Duncan's multiple range test)

product, malondialdehyde. This malondialdehyde is a reactive aldehyde that causes many toxic stresses in cells and tissues, and can be explained as an index of lipid oxidation (Dipierro and Leonardis, 1997; Eum et al., 2009; Kaniuga and Michalski, 1978). Malondialdehyde generally tends to increase as harvest period is extended. When malondialdehyde content is already present in the fruit tissue at harvest time, it is likely to be increased further throughout distribution and storage. Comparison of malondialdehyde content among five everbearing strawberries showed that it was the lowest in Flamenco, while it was far higher in Seascape compared to four other cultivars (Fig. 2B). The amount of malondialdehyde contained in Seascape was generally higher than in Flamenco and Goha during storage.

Effect of precooling during cold storage for flamenco and goha strawberries

Room precooling and forced-air precooling, which were treated to maintain the quality of strawberry berries during

Fig. 4 Change in soluble solids content (**A**, Flamenco; **B**, Goha) and titratable acidity (**C**, Flamenco; **D**, Goha) of everbearing strawberries during storage at low temperature (4 °C). The data presented the

mean $\pm$ SE ($n = 5$). The different normal letters indicate significant differences at 0.05 level (Duncan's multiple range test)

storage, resulted in weight loss during storage (Fig. 3). On the contrary, the control had less water loss during storage in Flamenco and Goha. Flamenco had the highest weight loss during storage by room precooling. Meanwhile, Goha showed a similar weight loss in both room precooling and forced-air precooling and was higher than the control. However, the weight loss of both cultivars with and without precooling was only about 6% after 12 days of storage. Furthermore, with only packing in the corrugated boxes without film packaging treatment, the weight loss could be minimized. Flamenco had higher firmness than Goha (Fig. 3). Precooling was effective in maintaining firmness in Flamenco. However, in the control, firmness was significantly decreased as storage progressed compared to the treated fruits. It indicates that forced-air precooling was more effective than room precooling. A similar tendency was observed in Goha, whose firmness was maintained by forced-air precooling. Generally, June-bearing strawberries cultivated mainly during winters show a firmness lower than 1 N, while the firmness of everbearing strawberries is around 2 N which is higher than that of June-bearing

strawberries (Choi et al., 2013). The texture of everbearing strawberry which is not easily softened is suitable for the decoration of cakes. Therefore, firmness maintained by precooling would be a good attribute to maintain the quality of the strawberries during storage and distribution.

In the case of Flamenco, SSC was gradually increased initially from 4° Brix in room precooling and there was no significant change throughout the storage period. However, the increase of SSC was higher in the forced-air precooling (Fig. 4). In the case of Goha, SSC did not show a constant tendency and there was no significant difference found between treatments. Overall, Goha tended to have a higher SSC than Flamenco, which is consistent with previous research results (Eum et al., 2014; Kallio et al., 2000). It is difficult to conclude that the SSC of everbearing strawberries is an important quality attribute as a whole. For the June-bearing strawberries, which are mainly used as fresh fruit, the average SSC is 11.4° Brix, Red Pearl is 9.6° Brix, and Akihime is 10.6° Brix (Chio et al., 2013; Jeon et al., 2013; Kim et al., 2004). On the contrary, since everbearing strawberries are cultivated mainly for decoration purposes,

Fig. 5 Change in hue angle (A, Flamenco; B, Goha) and chroma value (C, Flamenco; D, Goha) of everbearing strawberries during storage at low temperature (4 °C). The data presented the mean $\pm$ SE

(n = 5). The different normal letters indicate significant differences at 0.05 level (Duncan's multiple range test)

firmness acts as an important parameter and the SSC 4°–6° Brix would not be an important quality attribute (Eum et al., 2014). Generally, quality is an important factor to decide the quality of the commodity. The SSC of everbearing strawberries might not be important as in the winter strawberries.

The titratable acidity of Flamenco is almost the same as that of pretreatment between treatments (Fig. 4). In the case of room precooling, the SSC of strawberry fruits were slightly increased throughout storage. The changes in the SSC were similar in the forced-air cooling and control. Goha showed a higher acidity than Flamenco. However, there was no difference in titratable acidity according to storage pretreatment. The titration acidity at the initial stage of storage was maintained until a lapse of 12 days of storage. Horticultural crops continue to respire as organisms even after harvest. Organic acid is used as a respiratory substrate and tends to decrease during storage. However, in this experiment, organic acids were maintained until the end of storage. Therefore, it is considered that the storage condition is not a condition that causes an

increase in respiration. During the storage period, the sugar acid ratio of the everbearing strawberry ranged from 5.5 to 7.5 in Flamenco. Goha showed the sugar acid ratio of 6.2–7.9, indicating that it has high sugar acid ratio. This means that Goha has a better taste than Flamenco. In the case of apples with high sweetness, the sugar acid ration is known to be about 40. In general, the sugar acid ratio of June-bearing strawberry cultivars is 14.9 for Maehyang, 11.2 for Red Pearl, and 14.3 for Akihime, implying high sugar acid ratio compared to the everbearing strawberries in summers. The reason why everbearing strawberries have a low sugar acid ratio compared to the June bearing strawberries in winters is that titratable acidity is around 0.2%, which is higher in the everbearing strawberries. However, the SSC of June bearing strawberries are 11.4° Brix for Maehyang, 9.6° Brix for Red Pearl, and 10.6° Brix for Akihime, which is higher by more than 7° Brix compared with the everbearing strawberries (Kim et al., 2004). This is a major factor why everbearing strawberries cannot be used as fresh fruit. If sugar acid ratio is high in the

Fig. 6 Change in total phenolic content (**A**, Flamenco; **B**, Goha) and malondialdehyde (**C**, Flamenco; **D**, Goha) of everbearing strawberries during storage at low temperature (4 °C). The data presented the

mean $\pm$ SE ($n = 5$). The different normal letters indicate significant differences at 0.05 level (Duncan's multiple range test)

everbearing strawberries, these cultivars also could be used for fresh fruit as the June-bearing strawberries.

Chromaticity of everbearing strawberry was measured in terms of CIE L^* , chroma, and hue angle. Initial CIE L^* value of Flamenco was maintained throughout storage regardless of pretreatment (data not shown). In the case of Goha, L^* value was gradually decreased as storage period progressed compared with the initial storage. There was no difference in L^* of stored fruits by pretreatment. Hue angle refers to color. When the fruits are harvested for the first time, it was 40° for Flamenco and 45° for Goha, showing that Flamenco was harvested at the stage of deeper red color. Hue angle change during storage shows similar tendency to CIE L^* . Flamenco maintained the hue angle around 40° from the beginning of storage till the end of storage, but there was no significant difference between storage pretreatments (Fig. 5). However, the hue angle of Goha was gradually decreased and showed around 35°, showing deeper red. There was no difference in the hue angle by pretreatment. The chroma value that can confirm

saturation of the fruit color did not show a significant difference between two cultivars or storage period (Fig. 5).

During the storage period after the precooling, the functional components of everbearing strawberry were identified by measuring the total phenolic content and malondialdehyde. In Flamenco, the total phenolic compound tended to decrease with storage period (Fig. 6). The storage pretreatment tends to maintain steady state until tenth days of storage in case of forced-air precooling. Meanwhile, the total phenolic compound was increased or decreased without showing a constant tendency until a lapse of 10 days of storage in the room precooling and control. Meanwhile, the effect of storage pretreatment was maintained at its highest in the control of Goha after a lapse of 2 days of storage. It showed large decreases in the forced-air precooling. However, there was no clear difference afterwards between treatments. This indicates that a storage condition of temperature lower than 4 °C for Goha is an optimum condition to maintain the quality of everbearing strawberries by maintaining functional composition. Reactive oxygen species (ROS) decomposes

unsaturated fatty acids present in the cell membrane and produces a final product, malondialdehyde. This malondialdehyde is a reactive aldehyde, which causes many toxic stresses in cells and tissues. Therefore, increased production of malondialdehyde during storage may be explained by the fact that the cells and organs are stressed during the storage period and the membrane is largely damaged much (Shao et al., 2005; Smith and Lands, 1972). In this study also, malondialdehyde was increased in two cultivars of all everbearing strawberries after 4 days of storage compared to the initial stage of storage (Fig. 6). Flamenco showed the least increase in malondialdehyde until 8 days of storage in the forced-air precooling treatment. Malondialdehyde was increased in the order of the control and room precooling. In Goha also, malondialdehyde was increased at a span of 4 days of storage and maintained until the final stage of storage. However, there was no significant difference in malondialdehyde contents between pretreatments. The magnitude of increases in malondialdehyde was higher in Flamenco compared to Goha as a whole.

Taken together, these results suggest that cold storage of everbearing strawberries can be accomplished even with only a corrugated box packaging at 4 °C until 12th day of storage. Precooling treatment before storage was effective in maintaining firmness, a key quality attribute of everbearing strawberries during cold storage. In addition, total phenolic compounds decreased throughout storage period. Forced-air precooling might have been effective in maintaining the total phenolic compound and the effect was prominent by the cold storage. Still, no significant difference was found between pretreatments for other compositional aspects of the strawberries.

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